

Tropical statement

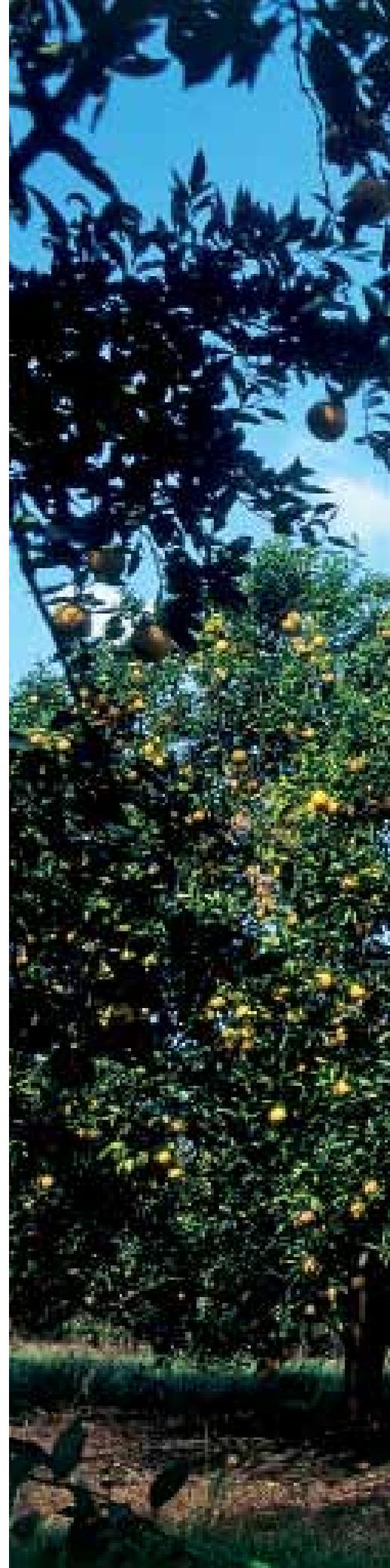
Brazil's pioneering foray into genomics
unveiled its capacity for scientific production
and is generating knowledge to this day

Mariluce Moura

X*ylella fastidiosa* has become by now an old acquaintance of biologists and Brazilians in all fields of activity who read up on the country's scientific achievements. But the extensively researched disease caused by this bacterium in orange trees and other citrus plants – citrus variegated chlorosis (CVC), better known in Brazil as *amarelinho* – continues to parry the attacks by the persistent scientists who have been working for more than a decade to fully decipher it. “We know how the disease starts and how the bacterium occupies the plant's vascular tissue and grows there, but we are unable to effectively control the establishment of the disease,” says Marie-Anne Van Sluys, a full professor in the Botany Department at the University of São Paulo (USP) and head of the Genomics and Transposable Elements Laboratory (GaTE-Lab) at the same university. However, because the scientific method is not deterred by lack of knowledge and its followers are allowed to simultaneously propose various hypotheses for the same problem – in other words, to strike it from different angles – there is today also a “strong perception that the key moment for controlling the disease is when the bacterium begins to produce the biofilm,” Van Sluys continues.

This biofilm, which received little attention during the meteoric rise to fame of *X. fastidiosa* as the

Orange grove
in state of São
Paulo: citriculture
is an economic
mainstay of Brazil's
richest state







Cover of *Nature* on July 13, 2000: international recognition for the Brazilian project

masse and in a short amount of time, greatly expanding the molecular biology research capabilities of São Paulo and Brazil as a whole. And the third was to mobilize the scientific community to study significant socio-economic problems that it could help to solve, such as citrus variegated chlorosis, very worrisome at the time. Amidst the discussions taking place on the fourth floor of the FAPESP headquarters, few could have guessed the hefty dose of daring, self-confidence and persistence that would be required over the coming months from those people and institutions who were about to brave the uncharted territory of genomics in Brazil. “The was an enormous chance of it all going wrong,” recalls with a smile the physicist José Fernando Perez, now an entrepreneur, then the scientific director at FAPESP and one of the greatest driving forces behind the amazing leap that the *X. fastidiosa* project represents for molecular biology in Brazil. “I bet no other genome sequencing project in the world started out so ill-prepared,” the project’s DNA coordinator, British researcher Andrew Simpson, said good-humoredly after all was said and done.

On February 21, 2000, having successfully and exultantly completed the *X. fastidiosa* genome sequence 28 months after the official announcement, the project’s 192 researchers basked in a staggering round of applause from more than a thousand people gathered in the gorgeous *Sala São Paulo* orchestra hall to celebrate the feat. Trophies, medals and certificates of scientific and technological merit were passed around, created especially for the occasion by the São Paulo state government (let it be noted: the longest ovation of the evening was reserved for Perez, hailed as the conductor of a masterpiece).

Two years and nine months after the launch of an initiative harshly criticized by those who claimed that genome sequencing was not science – albeit far outnumbered by advocates of the scientific leap it could represent for the country –, on July 13, 2000, the scientific journal *Nature* awarded its cover story to the *Xylella* project. Issue 6,792, volume 406 of the journal published not only the seven-page paper containing the project’s original findings, signed by 116 scientists, but also a detailed *News and*

star of Brazil’s groundbreaking genomic research project in 2000, is a gelatinous material deposited on the cell walls of the host plant’s vascular tissue, right next to another substance that the bacterium also produces: xantham gum. Conversely, on account of its visible role in the clogging and consequent obstruction of plant xylem, preventing the free passage of water and nutrients, xantham gum was prominently acknowledged as an important element in the characterization of CVC, in the scientific paper in which the complete genome of *X. fastidiosa* was published. “We now feel that if biofilm production were to be interrupted when the bacterium starts to produce it, or if that production were somehow to be compromised, we would be in a good position to control the disease,” says Van Sluys, currently an adjunct coordinator of FAPESP Life Sciences.

Controlling the bacterium would be a desirable outcome – a cherry on top, so to speak –, but it was not a central target of the *Xylella fastidiosa* genome sequencing project, justifiably portrayed at the time of its announcement as an endeavor destined to revolutionize Brazilian science. On that morning of October 14, 1997, the FAPESP auditorium was packed to the rafters. There the professors, researchers and businesspeople in attendance

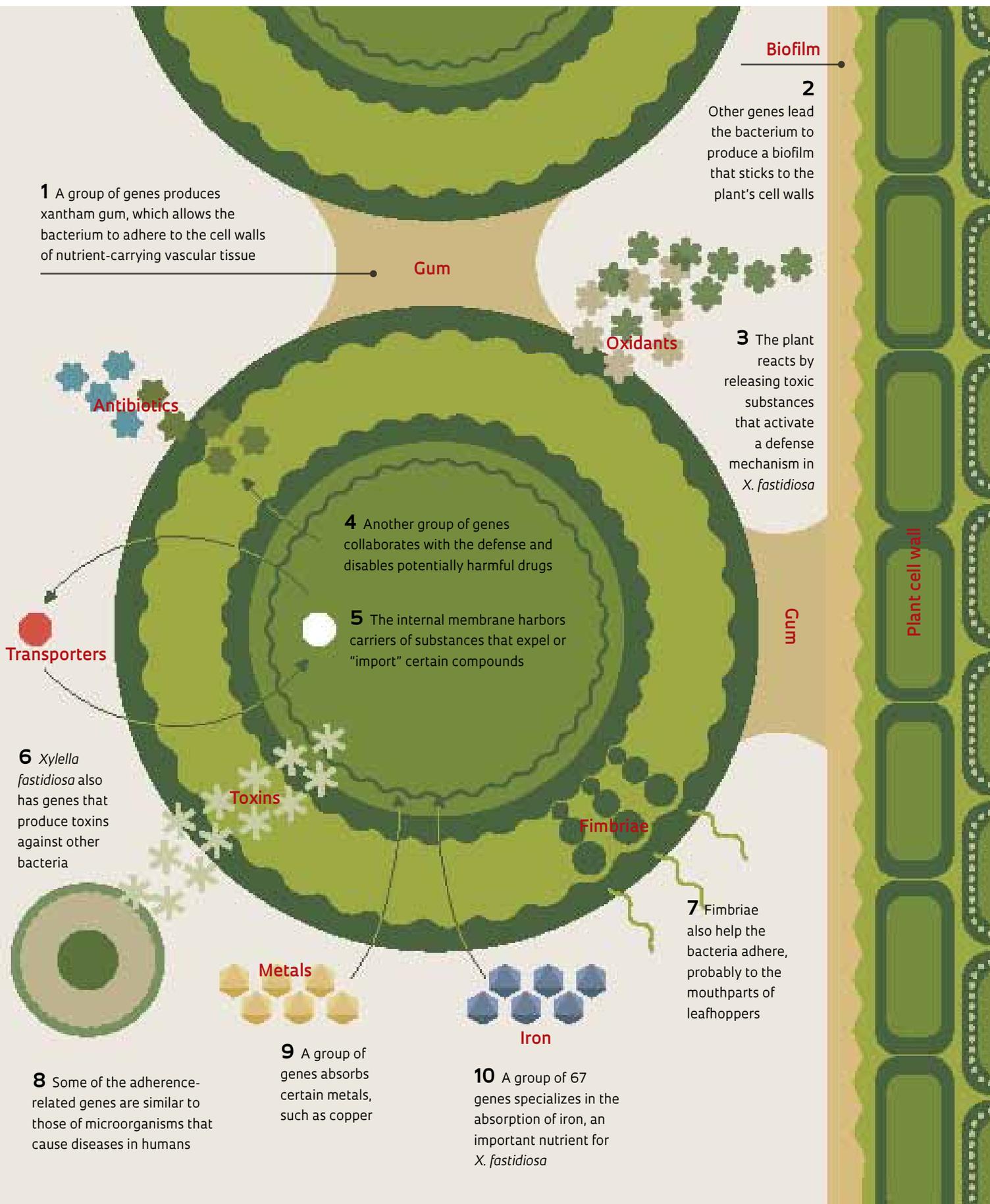
The key moment for controlling the disease may be when the bacterium begins to produce biofilm

received a detailed account of how the ambitious initiative would receive US\$12 million from the Foundation – an astonishing sum for a single research project at the time –, plus another US\$650 thousand from the Citriculture Defense Fund (Fundecitrus). Its primary goal would be to fully sequence the pathogen’s genome and describe the new scientific findings uncovered by the process. In other words, it was science at the leading edge, bringing a molecular biology project to a successful conclusion.

The project’s second objective was to train highly qualified researchers, *en*

Xylella fastidiosa and the orange tree

The bacterium has 2.7 million base pairs and 2,904 genes, and Brazilian researchers have successfully identified what 47% of them do





The leafhopper that transmits citrus variegated chlorosis

Views story about the study, plus an editorial in which Brazil's groundbreaking genomics project is called "as much a political achievement as a scientific one".

It was an accurate definition. First of all, the *X. fastidiosa* project has unmistakably produced scientific knowledge that continues to bear fruit 12 years after the bacterium's genome was sequenced. At the same time, it has accomplished its announced purpose of quickly expanding Brazil's capabilities in molecular biology and served as a stepping stone for a number of other genomics projects launched within the FAPESP Genome Program. And what's more: the project helped put Brazil on the map of international scientific production and was a decisive factor in redesigning the country's image in the local and international media, in terms of its ability to create scientific knowledge. As if the political dividends were not enough, the project's architecture, the pace at which it progressed, and the use of a new medium for the exchange of information and data among researchers in more than thirty separate laboratories (a.k.a, the internet) all had an obvious impact on how science has been produced in São Paulo since then, spurring it into contemporary speed. Finally, the project also gave rise to the formation of companies to do genomics-related business, spin-offs that have since been absorbed by the market.

Above all, the project helped put Brazil on the map of international scientific production

Let us now recollect some essential milestones of the *Xylella* experiment. A good place to start would be the contributions to genomics mentioned in the *Nature* article and featured in *Pesquisa FAPESP* issue No. 55, of July 2000. The original paper reports the project as the 24th complete bacterial genome known to science and the first from a phytopathogen. The sequencing revealed that the investigated microorganism has one chromosome with 2,679,305 nitrogenous base pairs or nucleotides.

That chromosome carries 2,904 genes, or protein coding regions, one third of them new to science in the year 2000.

Out of the total number of genes, the Brazilian group successfully described the functions of 47%, a slightly lower percentage than what other bacterial genome research groups were able to puzzle out – for instance, 54% of all gene functions were described for *Thermotoga maritima*, 52.5% for *Deinococcus radiodurans*, and 53.7% for *Neisseria meningitidis*. The authors attributed their lower score to the absence of any other completely sequenced genome of a phytopathogen bacterium.

At the turn of the century, when Brazil was producing nearly half of all the orange juice concentrate sold worldwide, the local citriculture industry earned up to US\$2 billion per year; export revenues were around US\$1.6 billion per year; and citrus-related activities accounted for 400 thousand direct and indirect jobs in the state of São Paulo. In that scenario, an estimated US\$100 million was being lost every year on account of orange trees infected by CVC. By then, the disease was already present in 34% of all orange groves in the state of São Paulo. Data from 2009 reveal an expansion of the industry's total annual revenues to US\$6.9 billion, exports to the tune of US\$3.15 billion, far fewer people directly or indirectly employed – more specifically, 230 thousand –, and chlorosis afflicting an identical proportion of commercial orange groves in São Paulo, i.e., 35%.

The goal was to ally science with production; to bring science closer to Brazil's GDP, national wealth, and social and economic development

The *Nature* article describes the fine-tuned metabolism of *X. fastidiosa*, with its adaptations that allow it to use the free sugars found in xylem sap and to obtain glucose by breaking down the cellulose in the host's cell walls. Among the project's most important discoveries were the specific genes that code the molecules involved in bacterial adhesion. These molecules, previously seen only in pathogens of humans and other animals, are found on the surface of the bacterium's cells, effectively gluing it to the epithelial tissue of host species. Finding these molecules in *X. fastidiosa* added to the evidence that bacterial pathogenicity mechanisms are the same regardless of whether they infect plants, animals, or human beings.

The recent impacts of the hypotheses and observations made at that time have proven them right, and new discoveries have followed suit. The research group headed by Marcos Machado and Alessandra de Souza at the São Paulo Agency for Agribusiness Technology - Campinas Institute of Agronomy (Apta-IAC) identified the existence of persistent cells in the vascular tissue of plants, which is

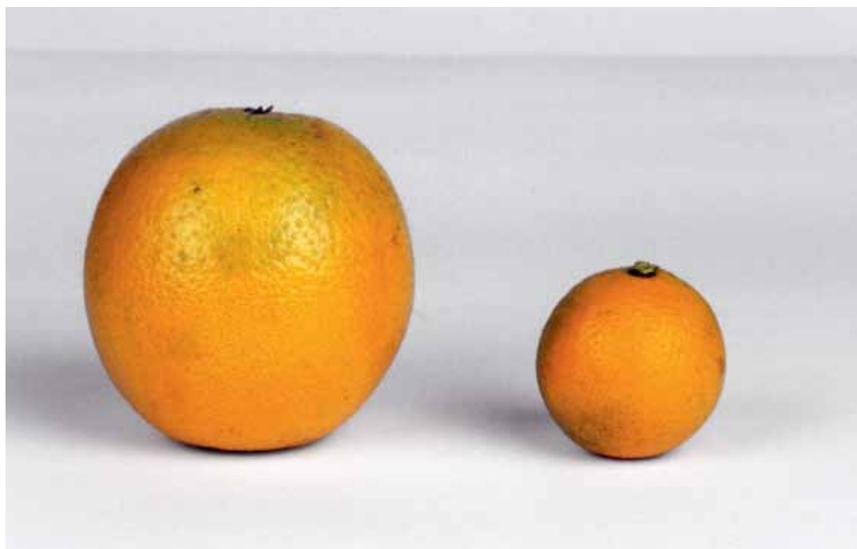
partly to blame for the difficulty of controlling CVC and its persistence in the field. The researchers also demonstrated that the bacterium goes through two stages in life: the planktonic phase and the biofilm phase. Further work on the topic was done by the group led by Aline Silva at the Chemistry Institute of the University of São Paulo (IQ/USP), showing not only the importance of the bacteria's adherence to plant vascular tissue, but also of iron signaling, adding weight to the prior discovery of 67 genes involved in the uptake of iron and other metals from plant sap.

The achievements of the Brazilian researchers are clearly summarized in the final conclusions of the *Nature* article. They have determined “not only the basic metabolism and characteristics of the bacterium's replication, but also numerous potential mechanisms of pathogenicity. Some have never before been postulated for phytopathogens, providing new perceptions on the generality of these processes.” According to the paper, the results will engender a detailed comparison between plant and animal

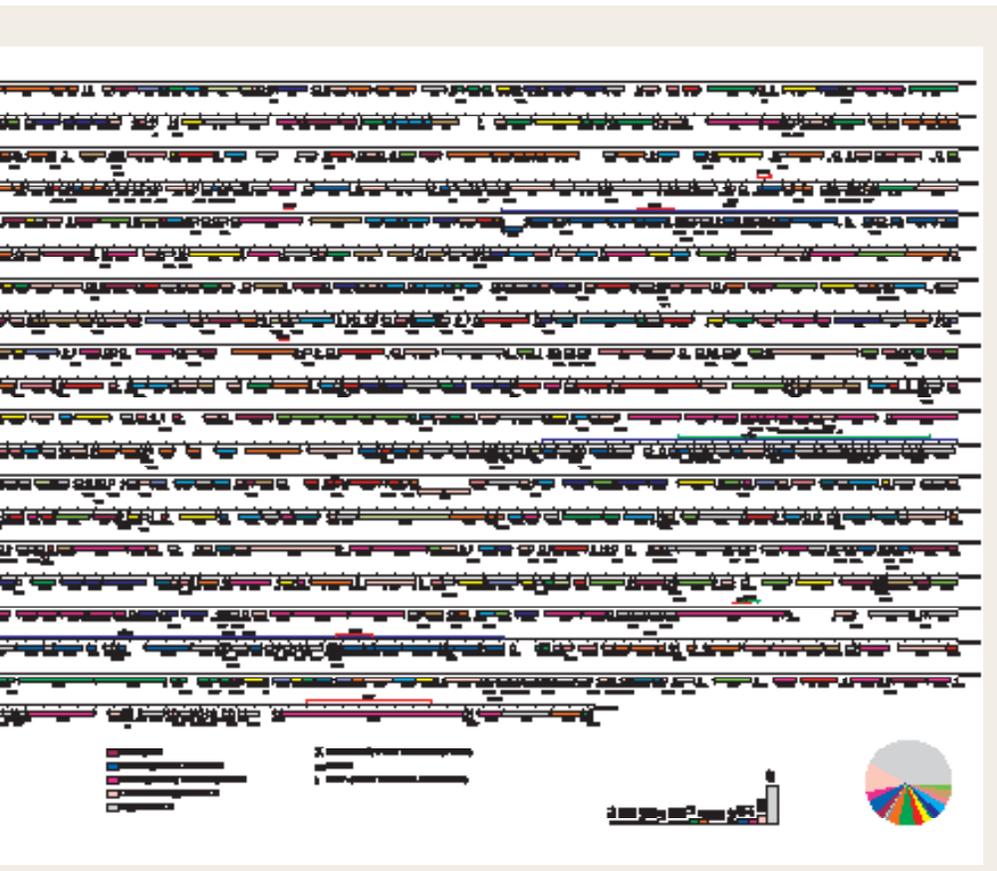
pathogens. And, to wrap up the story, “the new information should provide bases for an experimental, accelerated and rational investigation of the interactions between *X. fastidiosa* and its hosts, which should lead to new findings for the control of CVC, the infamous *amarelinho*.”

The terminology used in that paper was still the stuff of dreams when, upon opening the ceremony on October 14, 1997, physicist Carlos Henrique de Brito Cruz, then-CEO of FAPESP and now the Foundation's scientific director, classified the *Xylella* genome as “a unique project, destined to earn a place in the history of science and technology in the state of São Paulo and Brazil”. He justified his prediction on with arguments that compose a line of thought that Cruz has been refining over the years, on the construction of knowledge societies. He observed that the project would take aspects of basic science and leading-edge theoretical studies and merge them with applied research and technological development. “With this project, they will do something that has been a central concern for FAPESP and other science and technology institutions in São Paulo and Brazil: ally science with production; bring science closer to the GDP, to national wealth, to social and economic development.” Cruz continued along those lines, finally classifying the project as “revolutionary.”

The origins and architecture of the *X. fastidiosa* initiative were presented to the public by José Fernando Perez, FAPESP scientific director at the time, but not before he had summoned up a few figures to support his view that setting up a project of such proportions was urgent and indispensable. Those numbers, newly released by the Ministry of Science, Technology and Innovation, indicated that while Brazil's overall participation in global scientific production – as recorded in the Institute of Scientific Information (ISI) database – had practically doubled from 1981 to 1995, from 0.44% to 0.82%, the country's performance in molecular biology had advanced much more slowly. In other words, whereas



The smaller orange was picked from a tree infected by *Xylella*



making process that drives the production of knowledge and brings change to the scientific production environment, were briefly mentioned in the opening ceremony. It was only later on, retold by Perez or Reinach, that they would become elements in the history of what appears to be the most extensively covered Brazilian research project, both inside and outside the country.

“We needed a new idea that would change Brazilian biotechnology, creating capability,” Perez recalled during the lecture in 2008. He perceived it as a strategic area with the ability to address “the country’s specific economic characteristics, biodiversity, agriculture, livestock industry, and public health problems.” Such was the tenor of his discussions with advisors in early 1997, especially Fernando Reinach, then a full professor of biochemistry at the University of São Paulo (USP) and a coordinator under the FAPESP scientific director. And it was a continuation of one such discussion that led Reinach to make a fateful phone call to Perez.

They sought an idea that would change Brazilian biotechnology, creating capability

“On the weekend of May 1, 1997, I was at my country house in Piracaia and I thought: instead of having an infrastructure project, let’s do a genome project, bringing together everyone under a single objective. For me this was a very strange idea. I phoned Perez, who was in Santos, and he came to Piracaia. We talked and the idea crystallized.”

On the week right after the holiday (May 1 is Labor Day in Brazil), Perez began pulling strings to organize the project. The microorganism to be sequenced had not yet been chosen, but would certainly be a bacterium with a genome big enough to allow a large number of peo-

ple to get involved in the project, but small enough to keep it feasible. From the United States, where he was working three months out of the year at the time, Reinach soon sent Perez the project’s first draft, as he had requested. According to the former scientific director of FAPESP, all of the fundamental ideas that would make up the project’s backbone were already there.

The discussions about the project gradually came to incorporate new protagonists, at the same time as the topic was being debated by the FAPESP board of trustees. Paulo Arruda, a biologist and professor at the State University of Campinas (Unicamp), and Marcos Machado, from the Sylvio Moreira Citriculture Center, were among the first to join the discussions. Later on, Ricardo Brentani suggested that Perez expand the group to include Andrew Simpson, a British researcher who had been working in Brazil for a number of years. The project had reached the stage where its characteristics were being worked out, and it was time to call

in some big-league international consultants. Paulo Arruda brought in André Goffeau, whose qualifications included extensive experience in bioinformatics. In a genome project, bioinformatics is a key area that could have gener-

ated critical problems at the time, given Brazil’s virtually nonexistent expertise in the required techniques. One of the ideas proposed by Goffeau was to have that part of the study be conducted in France. But Reinach suggested that the group have a talk with two young researchers from the Institute of Computing at Unicamp, João Setúbal and João Meidanis, who had some experience with bioinformatics applied to genome simulations and had published a book on the topic, although they had never worked on a real genome. “So there was some uncertainty in the air. The international consultants did not believe we

could solve the bioinformatics problems, but that eventually proved to be one of the project's greatest successes," Perez remarked in one of his later assessments of the *Xylella* initiative.

Aside from Goffeau, the steering committee of international advisors included two Englishmen: Steve Oliver and John Sgouros. And it was during a meeting with Oliver at FAPESP that the project's command structure was defined: there would be two central laboratories to which all other laboratories involved in the sequencing work would report, plus a bioinformatics laboratory and a general coordinator. The request for proposals, released soon after the project's official announcement, followed those guidelines when defining the available openings to be filled by the laboratories. And so Andrew Simpson became the project's general DNA coordinator, with responsibilities including making sure that bacterial clones were delivered to every laboratory. Reinach and Arruda were chosen as sequencing coordinators and Meidanis and Setúbal became the bioinformatics coordinators.

According to Reinach, to select 30 laboratories out of the nearly one hundred that replied to the tender, the criteria were that 10 would have to have some prior knowledge of sequencing, another 10 would need to include researchers coming from agriculture and who knew a thing or two about *Xylella*, and 10 had to be headed by people who did not know the bacterium, had no sequencing experience, but "had unquestionable scientific competence and a permanent desire to learn more; the profile, for example, of José Eduardo Krieger."

In an interview for *Pesquisa FAPESP* after the *Xylella* sequencing was complete (Special feature "The future of genomics in Brazil", issue No. 51, March 2000), Andrew Simpson, proud of his role in the project and having profusely celebrated the fact that Brazil "gate-crashed an exclusive party and made a good showing", gave a detailed account of how difficult it had been to overcome the generalized lack of knowledge about *X. fastidiosa*. With perpetual and refined good humor, Simpson recalled some of the other obstacles that presented themselves once the work got underway. "There was this one coordinator who had never sequenced a genome,

A PubMed search will reveal more than 300 papers citing *X. fastidiosa*, more than 100 of them Brazilian

had never worked on bacteria, and didn't even know what *Xylella fastidiosa* was. There was an informatics coordinator who had never touched a DNA project in his life and whose team was scattered all over the state of São Paulo. There were several who knew almost nothing about molecular biology. And what's worse: nobody in the entire state had the live bacterium, let alone its DNA, much less the DNA library. We started out with no evidence whatsoever that the project would work." According to Simpson, it was Joseph Bové's promise that he would provide the bacteria and the necessary DNA that gave them the confidence to get started. "Ultimately, it was Marcos Machado from the Campinas Institute of Agronomy (IAC) who provided all the DNA, which I then used in the project."

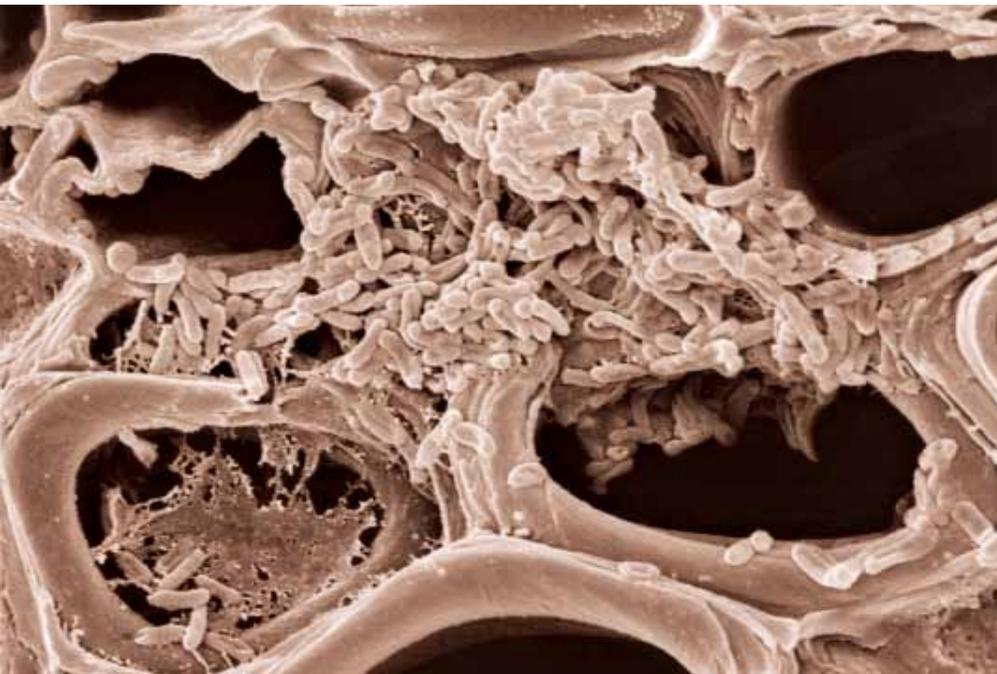
Simpson freely admits that conditions at the outset were less than ideal for accomplishing what had been announced, yet he did not consider that initial stage "Brazilian madness", not even taking into account that the project relied heavily on an internet connection that was still woefully precarious at the time. "There was confidence in the community's ability to execute any task at hand. There's nothing crazy about it." According to Simpson, the toughest moment of all was the final stage, when the time came to close the gaps left in the assembled sequence. "First, I hadn't the slightest idea how to go about it. I had never done it before and the papers don't tell you exactly how to proceed. It was necessary to invent a solution. Second, we had very little information about *Xylella* before the sequencing started. We didn't even know the size of the gaps, because we didn't know the total size of the genome." So, as Marie-Anne Van Sluys observed at the time, when the last pieces of the giant jigsaw puzzle fell

into place on January 6, 2000, one word became a symbol of victory, a codeword of sorts repeated over the phone as the good news spread to 35 laboratories all over the state of São Paulo: "*Fechou!*". In other words, "We closed it!"

Brazil's giant leap forward, definitively leaving behind the days of slow, awkward handling of the sequencing machines imported in early 1998, was finally wrapped up by researcher Luís Eduardo Aranha Camargo as he submitted the final read (part of the clone library) that would close the genome. And the public found out about it when Simpson announced the project's successful completion to the scientists gathered at the 1st Agricultural Microbes Genome Conference, sponsored by the U.S. Department of Agriculture in San Diego, California, on January 8 and 9, 2000. Brazil had gained a type of expertise mastered until then by only 14 other research groups in the United States, Europe, and Japan.

Today, a simple search for *X. fastidiosa* covering the period 1995/May 2012 on PubMed, a leading reference for high-quality publications in the medical field, will return 343 articles citing the bacterium, 330 of them published after the Brazilian paper that appeared in *Nature*. One hundred and ten of those articles, i.e., one third of the total, were authored in Brazil, making a notable contribution to the available knowledge on the topic.

"Over the years, the predictions and hypotheses laid out in our first paper were shown to be correct," says Van Sluys. The current question is the origin of CVC and the role of the biofilm in the disease. "We have strong reason to believe that the disease could be controlled by intervening with the formation of the biofilm," she continues. A patent has been deposited by the group coordinated by Marcos Machado and Alessandra Alves (INPI: 018110011623 PCT: BR/2012/000003 Deposit date: INPI



Xylella colony infests and clogs the walls of orange tree xylem, which carries water and nutrients

ELLIOT W. KITAJIMA/USP

03/31/2011 PCT: 1/9/2012), in which a cysteine analog is used to block that process, a strategy already applied successfully for human pathogens. Furthermore, given the enormous amounts of viruses detected in the genomes of several different strains of *Xylella*, as observed in the microarray expression studies carried out by the groups headed by Dr. Suely Gomes (IQ-USP) and Dr. Marilis do Vale Marques (ICB-USP), jointly with the sequencing of multiple strains of *Xylella* coordinated by Dr. Aline Silva, it appears that environmental conditions may induce viral multiplication to a point where the bacteria are killed. “If the virus splits off from the genome and starts propagating by making viral capsids, it can explode the cell and kill the bacterium,” she imagines.

In the environment so richly fertilized by Brazil’s first genomics project, the research teams that consolidated themselves in this country formed new leaderships, established solid international collaborations, and they are now asking themselves about that and much more. For example, the group originally led by Marcos Machado at IAC, now with Alessandra Alves de Souza captaining the *Xylella* research line, is tackling the notorious bacterium’s interaction with its host plant. The group led by Marilis do Valle Marques has been working to obtain *X. fastidiosa* mutants – which can be useful for Aline Maria da Silva at the

USP Biochemistry Department – and is heading the genomic study on different strains of *X. fastidiosa* in South America, three of them citrus pests and the others responsible for coffee, plum, and hibiscus diseases, thus advancing the research on *Xylella* and its pathogenic genes.

In addition to the continued research, other confirmations of the *Xylella* project’s success are also worthy of note, such as the three companies created by researchers involved in the project: Alellyx, which offers both genomic resources and other biotechnological tools for agricultural applications; Scylla, a bioinformatics company; and CanaVialis, which specializes in new varieties of sugarcane. The first and third were acquired by their main investor, the Votorantim Novos Negócios fund, also created in the wake of the pioneering genomics project and directed by Reinach. Then, in 2008, the two companies were purchased by Monsanto.

The *Nature* editorial on *X. fastidiosa* in 2000 observed among other things that the project’s success, plus the unusual fact that an agency from the developed and industrialized world – the U.S. Department of Agriculture – had commissioned genomic research on a *Xylella* variant from a developing country, “endorses Brazil’s determination to enter the post-genomic age hand in hand with scientists from the wealthiest countries”. ■

PROJECTS

1. 2. 3. and 4. *Genome Project* – FAPESP: Sequencing Laboratory – No. 1997/13451-6, 1997/13457-4, 1997/13475-2, and 1997/13463-4 (1997-2000)

GRANT MECHANISM

1. through 4. Genome Program

COORDINATORS

1. Andrew John George Simpson – Ludwig Institute
2. Fernando de Castro Reinach – USP
3. Paulo Arruda – Unicamp
4. Jesus Aparecido Ferro – Unesp

INVESTMENTS

1. R\$1,329,975.72
2. R\$1,535,926.46
3. R\$932,244.71
4. R\$1,534,700.66

SCIENTIFIC ARTICLES

SIMPSON, A.J. *et al.* The genome sequence of the plant pathogen *Xylella fastidiosa*. The *Xylella fastidiosa* Consortium of the Organization for Nucleotide Sequencing and Analysis. *Nature*. v. 406, n. 6792, p.151-59, 2000.

Silva, A.C. R. da *et al.* Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature*. v. 417, n. 6887, p. 459-63, 2002.

FROM OUR ARCHIVES

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The first results
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